TITLE OF THE INVENTION

[0001] COMPOUNDS AND METHODS FOR MODULATING BACTERIAL FUNCTIONS

CROSS-REFERENCE TO RELATED APPLICATION

[0002] This application claims priority from U.S. provisional application no. 60/414,351, filed September 30, 2002, which is pending.

FIELD OF THE INVENTION

[0003] The invention relates to the modulation of bacterial functions and particularly to modulators of functions in biofilm producing bacteria.

BACKGROUND OF THE INVENTION

[0004] To persist in nature, bacteria must be able to compete and survive under varying growth conditions. To accomplish this task, they possess regulatory systems that permit them to recognize and adapt to a changing environment. In *Escherichia coli* and related species, the transition from exponential growth to stationary phase growth is accompanied by striking physiological changes, which produce cells that are more stress resistant, slower metabolizing, and better at scavenging nutrients. These adaptations are brought about largely through changes in gene expression that are coordinated through global regulatory networks.

[0005] Thus, it is an object of the invention to provide compounds and methods for modulating bacterial functions.

SUMMARY OF THE INVENTION

[0006] The present invention discloses interactions among different types of global regulatory systems that affect stationary phase gene expression, and uses and methods for modulating bacterial functions employing these interactions.

[0007] In an embodiment of the invention there is provided a modulator of biofilm formation in biofilm-producing bacteria.

[0008] In an embodiment of the invention there is provided a method of modulating biofilm formation by biofilm-producing bacteria.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIGURE 1 is a graphical representation of the results of northern analysis of CsrB levels in *E. coli* K-12 MG1655 and isogenic *barA* and *uvrY* knockout mutants. RNA from cultures harvested at 2 h post-exponential phase of growth was probed for the CsrB transcript. Panel A depicts a blot representative of the observed results. An overexposed film is shown to reveal CsrB RNA from the *uvrY* mutant. Panel B shows quantification of the signal by phosphorimage analysis. Signals were normalized with respect to MG1655, which was assigned a value of 100%. Each bar represents the mean value obtained from 2 independent experiments. Where error bars depicting the standard deviations (SD) are not apparent, the SD were below the resolution of the graph.

[0010] FIGURE 2 is a graphical representation of the effects of the *uvrY* or *barA* null mutations on expression from *csrA'-'lacZ* translational or *csrB-lacZ* transcriptional fusions. β-Galactosidase specific activities expressed from *csrA'-'lacZ* in strains KSA712 (wild type), UY KSA712 (*uvrY*::*cam*), and BA KSA712 (*barA*::*kanR*) (A), and from *csrB-lacZ* in strains KSB837 (wild type), UY KSB837 (*uvrY*::*cam*), or BA KSB837 (*barA*::*kanR*) (B) are shown. β-Galactosidase activities in the wild type, *uvrY*::*cam*, or *barA*::*kanR* strains are shown as closed circles,

squares, or triangles, respectively. Growth (*A*600) of the respective strains is depicted by open circles, squares, and triangles.

[0011] FIGURE 3 is a graphical representation of the effects of *csrA* and *uvrY* mutations on expression from a *barA-lacZ* transcriptional fusion. (A) β-Galactosidase specific activities expressed from a *barA-lacZ* fusion in strains CAG HS703 (wild type) and TR1-5 CAG HS703 (*csrA*::*kanR*) are shown as closed or open circles, respectively. (B) Activities in strains HS703 and UY HS703 (*uvrY*::*cam*) are shown as closed or open circles, respectively. In each panel, growth (*A*600) of the respective strains is depicted by closed or open squares.

[0012] FIGURE 4 is a graphical representation of the results of complementation studies: effects of ectopic expression of *csrA* (pCRA16), *uvrY* (pUY14) or *barA* (pBA29) on expression of a *csrB-lacZ* transcriptional fusion in isogenic *csrA*, *uvrY*, or *barA* mutants of KSB837. The vector control was pBR322 in each case. Specific β-galactosidase activities and growth (A600) at 24 h are shown as bars and closed circles, respectively. This experiment was repeated with essentially identical results.

[0013] FIGURE 5 is a graphical representation of the effects of the *csrB* or *uvrY* null mutations and *uvrY* overexpression on a chromosomal *glgCA'-'lacZ* translational fusion. (A) β-Galactosidase activities expressed from *glgCA'-'lacZ* in strains KSGA18 (closed circles) and UY KSGA18 (*uvrY*::*cam*) (open circles), (B) KSGA18[pBR322] (closed circles) and KSGA18[pUY14] (open circles), (C) RG KSGA18[pBR322] (*csrB*::*cam*) (closed circles) and RG KSGA18[pUY14] (*csrB*::*cam*) (open circles) (C) are shown. In each panel, growth (*A*600) of the respective strains is depicted by closed or open squares.

[0014] FIGURE 6 is a pictorial (A) and graphical (B) representation of the results of an *in vitro* transcription-translation of the *csrB-lacZ* transcriptional fusion carried on pCBZ1. Reaction mixtures contained pCBZ1 (*csrB-lacZ*) or vector only (1.6 μg), as indicated. Reactions were conducted in the absence or presence of UvrY protein, in an S-30 extract from UY CF7789 (*uvrY*::*cam*). (A) Labelled proteins were analyzed

by SDS-PAGE and fluorography. The position of an unlabeled standard of β-galactosidase (LacZ) is shown. (B) Incorporation of [^{35}S]-methionine into the LacZ polypeptide was determined by liquid scintillation counting. Approximately 5.5X10^3 cpm is equivalent to 1 pmol of LacZ polypeptide per reaction per h.

[0015] FIGURE 7 is a graphical representation of the effects of *sdiA* disruption and overexpression on expression of chromosomal *uvrY*-'*lacZ* and *csrA*-'*lacZ* translational fusions and a *csrB-lacZ* transcriptional fusion. Expression from *csrA*-'*lacZ* in (A) KSA712 and SA KSA712 (*sdiA*::*kanR*) and (B) KSA712[pBR322] and KSA712[pSdiA]. Expression from *csrB-lacZ* in strains (C) KSB837 and SA KSB837 (*sdiA*::*kanR*) and (D) KSB837[pBR322] and KSB837[pSdiA]. Expression of *uvrY*-'*lacZ* in strains (E) KSY009 and SA KSY009 (*sdiA*::*kanR*) and (F) KSY009[pBR322] and KSY009[pSdiA]. In each panel, β-Galactosidase activities in the wild type strain are shown as closed circles, whereas activities in *sdiA*::*kanR* or *sdiA* overexpressing strains are shown as open circles. Growth (*A*600) of the respective strains is depicted by closed or open squares. This experiment was repeated in its entirety, with essentially identical results.

[0016] FIGURE 8 is a graphical representation of the effects of *csrB, uvrY, barA* and *sdiA* null mutations and ectopic expression of *uvrY, barA*, and *sdiA* on 24 h biofilms grown in microtiter wells. (A) Biofilm formation by the parent strain MG1655 and isogenic mutants, as indicated. (B) Effects of increased gene dosage of *uvrY* (pUY14), *barA* (pBA29), *sdiA* (pSdiA) on biofilm formation by MG1655. (C) Same as (B), except in a *csrB* null mutant (RG1-B MG1655). Bars show the average and standard error of three experiments, with three samples per experiment. Double asterisks indicate statistically significant differences between strains of a given set ($P<0.01$).

[0017] FIGURE 9 is a schematic diagram depicting a summary of the regulatory interactions of CsrA/B, BarA/UvrY and SdiA. CsrA activates *csrB* transcription indirectly (20). This effect of CsrA requires functional UvrY, which directly activates *csrB* transcription. The effect of CsrA on *csrB* is mediated in part by activation of

barA expression, but apparently also involves a BarA-independent, UvrY-dependent mechanism, shown as [X]. UvrY also activates the expression of *barA*, in an autoregulatory loop. SdiA activates the expression of *uvrY*, and to a lesser extent, that of *csrB*. Finally, CsrB RNA binds to ~18 subunits of CsrA protein and antagonizes its regulatory effects in the cell (31, 45).

[0018] FIGURE 10 is a depiction of the RNA sequence of the *glg* leader toeprint, structure mapping and footprint results. The positions of the CsrA toeprint, as well as the *glgC* SD sequence and start codon (Met), are shown in bold type. Positions of cleavage by the single-strand specific probes RNase T1 (G specific) and Pb^{2+} in the absence of bound CsrA are indicated by filled arrowheads and filled circles respectively. The structure of the hairpin loop in the absence of bound CsrA is shown. Nucleotides in which bound CsrA increases (+) or decreases (-) cleavage are indicated. Numbering is from the start of *glgCAP* transcription.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The global regulatory system Csr (carbon storage regulator) represses a variety of stationary phase genes. The central component of this system, CsrA, is a 61 amino acid RNA binding protein. This protein inhibits glycogen biosynthesis and catabolism, gluconeogenesis, and biofilm formation, whereas it activates glycolysis, acetate metabolism, motility and flagellum biosynthesis in *E. coli*. The dramatic effect of CsrA on biofilm formation is mediated primarily through its regulatory role in directing glycogen biosynthesis and catabolism. Homologues of *csrA* exhibit a broad phylogenetic distribution in eubacteria and repress stationary phase genes of *Pseudomonas fluorescens*, genes involved in plant pathogenesis in *Erwinia carotovora*, and regulate genes involved in mucosal invasion by *Salmonella enterica*. The genome of *E. coli* K-12 was reported by Blattner (1997, ref. 7)

[0020] CsrA is capable of post-transcriptional repression or activation, depending upon the particular RNA target. CsrA binds to the untranslated leader of the *glgCAP* transcript, which encodes enzymes required for glycogen synthesis, at a site that

overlaps the *glgC* Shine-Dalgarno sequence and a second site within a hairpin that is located upstream of the Shine-Dalgarno sequence from nucleotides -52 to +34 (relative to the glgC initiation codon), as shown in Figure 10. Thus, CsrA blocks ribosome binding and inhibits the initiation of *glgC* translation. Inhibition of translation is believed to contribute to the observed destabilization of *glgCAP* mRNA by CsrA. CsrA positively regulates motility in *E. coli* by binding to and stabilizing the *flhDC* transcript, which encodes the subunits of a tetrameric DNA-binding protein ($FlhD_2C_2$) that activates the expression of genes involved in flagellum biosynthesis, motility, and chemotaxis. CsrA binds to the untranslated leader sequence of the flhDC transcript (see also Wei *et al.*, *Mol. Microbiol.* 40(1): 245-56 (2001).

[0021] A second component of Csr is the 366 nucleotide untranslated csrB RNA, which binds to ~18 CsrA subunits, forming a large globular ribonucleoprotein complex. In vitro transcription-translation studies of *glgCAP* expression and *in vivo csrB* disruption and overexpression studies have revealed that csrB RNA functions as an antagonist of CsrA, apparently by sequestering this protein. A highly repeated sequence element that is located in the loops of predicted csrB hairpins, and is related to the sequences involved in *glgCAP* recognition sites, is believed to mediate the binding of CsrA to csrB. The consensus sequence for the imperfect repeat is CAGGAUG. (See also Liu *et al.*, *J. Biol. Chem.*, 272 (28): 17502-10 (1997)).

[0022] The function of csrB RNA as an antagonist of an mRNA binding global regulatory protein offers a novel paradigm for post-transcriptional control by procaryotic regulatory RNA molecules. CsrA indirectly activates *csrB* transcription, indicative of an autoregulatory mechanism that determines the intracellular activity of CsrA without affecting its level.

[0023] HCN biosynthesis (*hcnABC*) and extracellular protease (*aprA*) in *P. fluorescens* CHA0 are regulated indirectly by GacA, a homologue of UvrY, via a post-transcriptional mechanism involving RsmA and RsmZ. PrrB RNA, a 132-nucleotide transcript in *P. fluorescens* F113 is itself regulated by GacS/GacA. The *barA* gene of *Salmonella enterica* positively affects the expression of *hilA*, which

encodes a regulator of genes of pathogenicity island 1 (SPI1). Mutations in *csrA* or *csrB* also affect *hilA* expression. Furthermore, *gacS* and *gacA* (alternatively called *expS* and *expA*) of *Erwinia carotovora* affect levels of RsmB.

[0024] The sensor-kinase BarA of *E. coli* was identified as a multicopy suppressor of an *envZ* defect in the expression of outer membrane proteins, and was shown to activate the transcription of *rpoS*, which encodes the stationary phase sigma factor RpoS or sigma^{S}. BarA is a member of the subclass of tripartite sensor kinases. These proteins consist of an N-terminal cytosolic domain, a canonical pair of transmembrane regions linked by a periplasmic bridge, a transmitter domain containing a conserved histidine residue, a central receiver domain with a conserved aspartate residue, and a C-terminal phosphotransfer domain with a conserved histidine residue. Tripartite kinases catalyze the phosphorylation of their cognate response regulators via an ATP-His-Asp-His-Asp phosphorelay. The *barA* gene appears to play a role in the virulence of uropathogenic *E. coli* in the urinary tract. BarA is a cognate kinase of UvrY, a response regulator of the FixJ family. The *uvrY* gene is co-transcribed with *uvrC*, which encodes a DNA repair enzyme, although UvrY plays no apparent role in DNA repair.

[0025] Increased *sdiA* gene dosage causes *uvrY* transcript levels to increase by about 10-fold. The *sdiA* gene encodes a protein of the LuxR family, the members of which contain one domain for binding N-acylated homoserine lactones (AHL) and a second domain for binding DNA. These proteins permit the bacterium to sense and respond to the resident microbial population by binding to AHL beyond a threshold concentration and activating or repressing the transcription of target genes, i.e. they mediate "quorum sensing". *E. coli* and *Salmonella* are not known to synthesize AHL and there are no apparent AHL synthase genes in their genomes.

[0026] Regulatory interactions of BarA/UvrY, CsrA/CsrB and SdiA of *E. coli* are disclosed herein. The UvrY response regulator of *E. coli* directly activates transcription of *csrB* and mediates the indirect effects of CsrA on *csrB*. The BarA sensor-kinase and the DNA binding protein SdiA also regulate *csrB* transcription,

apparently through effects on UvrY phosphorylation and *uvrY* expression, respectively. Autoregulatory loops characterize these systems. CsrA activates transcription of its RNA antagonist, CsrB, and UvrY stimulates expression of *barA*, which encodes its cognate sensor-kinase.

[0027] The RNA binding protein CsrA and the untranslated RNA CsrB constitute a post-transcriptional regulatory system that has profound effects on central carbon metabolism, motility and multicellular behaviour of *E. coli*. CsrA is autoregulatory, and indirectly activates transcription of the gene for its RNA antagonist, CsrB. The simplest explanation for this role of CsrA is that CsrA activates a transcriptional activator which the present invention is not limited to any particular mechanism, the results disclosed herein support this hypothesis, and delineate signalling circuitry by which CsrA activates *csrB* transcription, namely the BarA/UvrY two component signal transduction system.

[0028] The organization of the signalling circuitry that connects the CsrA/CsrB and BarA/UvrY regulatory systems was defined by several kinds of evidence. First, the steady state levels of CsrB RNA were extremely deficient in *csrA* (20) and *uvrY* mutants (Fig. 1). The in vivo expression of a *csrB-lacZ* transcriptional fusion containing the region from –242 to +4 bp of *csrB*, relative to the start of transcription, is also highly dependent upon *csrA* (20) and *uvrY* (Fig. 2). Since this fusion is capable of expressing only the first 4 nucleotides of the natural *csrB* transcript, the latter experiment establishes that UvrY activates *csrB* transcript initiation, and does not only stabilize CsrB RNA. A *barA* mutant is partially defective for CsrB accumulation and *csrB-lacZ* expression (Figs. 1, 2), but this effect of *barA* is considerably less severe than those of *csrA* (20) or *uvrY* mutations (Figs. 1, 2). Second, *barA*, *uvrY*, and *csrA* itself have no effects on the expression of *csrA* (Fig. 2). CsrA and UvrY each stimulate *barA-lacZ* expression ~1.5 to 2-fold (Fig. 3). Mutations in *csrA*, *barA* or *uvrY* itself did not significantly affect *uvrY'-'lacZ* expression. Third, complementation studies with multicopy plasmids showed that *uvrY* suppresses the defects in *csrB-lacZ* expression that are caused by *csrA*, *barA* or *uvrY* mutations (Fig. 4). A *csrA* plasmid clone suppresses the defects of *csrA* or

barA mutants, but has no effect in a *uvrY* mutant (Fig. 4). Finally, a plasmid clone of *barA* suppresses the *barA* defect, but does not affect *csrB-lacZ* expression in strains defective for *uvrY* or *csrA* (Fig. 4). Because ectopic expression of *csrA* has no effect on *csrB* if *uvrY* is defective, while ectopic expression of *uvrY* suppresses a *csrA* defect, these complementation experiments provide strong genetic evidence that the effects of CsrA on *csrB* transcription are mediated through UvrY. CsrA does not appear to affect *csrB* by regulating the expression of the *uvrY* gene, although it is a modest activator of the expression of its cognate kinase, BarA (Fig. 3). Fourth, purified CsrA protein failed to regulate *csrB-lacZ* expression in S-30 transcription-translation assays, indicating that CsrA indirectly activates *csrB* expression, while recombinant UvrY protein activated the same *csrB-lacZ* fusion ~6-fold (Fig. 6). The latter result is believed to represent the first biochemical evidence that UvrY or any of its homologues directly activates gene expression, and positions UvrY immediately upstream from *csrB* in a signalling pathway. Fifth, mutations in *uvrY* or *barA* result in a reduction of the expression of *glgC'-'lacZ* and *glgCA'-'lacZ* translational fusions (Fig. 5), which are repressed by CsrA and activated by CsrB. While these effects of *barA* and *uvrY* were modest, they were in agreement with the modest effects noted for CsrB. The relatively weaker effects of CsrB in comparison to those of CsrA have been observed thus far for all Csr-regulated genes. They are consistent with the finding that CsrB levels in the cell are sufficient to bind only ~30% of the CsrA protein, assuming full occupation of the ~18 CsrA-binding sites on CsrB. The relatively lower level of CsrB in the cell relative to CsrA may also account for the modest effects of BarA/UvrY, since UvrY effects on *glgCA'-'lacZ* were largely dependent on the presence of a functional *csrB* gene (Fig. 5). Sixth, comparisons of *sdiA* wild type, mutant, and overexpressing strains confirmed that SdiA activates expression of a *uvrY'-'lacZ* translational fusion (Fig. 7). In addition, *sdiA* was found to activate *csrB* expression through its effect on *uvrY*.

[0029] While the invention is not limited to any particular mechanism, the model shown in Fig. 9 presents the results of the examples herein, within the regulatory circuitry of the Csr system. The RNA binding protein CsrA is the key regulator of the Csr system, and indirectly activates the transcription of its RNA antagonist, CsrB,

~20-fold. Although CsrA binds to CsrB RNA, it does not alter its chemical decay rate, which has a half-life of ~2 min. The effects of CsrA on *csrB* expression appear to be completely dependent upon UvrY (Fig. 4), which is a direct activator of *csrB* expression (Fig. 6). While *barA* is involved in the circuitry, mutagenesis (Figs. 1 and 2) and complementation studies (Fig. 4) suggest that CsrA can activate *csrB* expression independently of BarA. BarA has been shown to phosphorylate UvrY, and activation of UvrY by BarA likely occurs by phosphorylation. In addition, CsrA may cause a second kinase to phosphorylate UvrY. The relative effects of CsrA, UvrY and BarA on *csrB* expression and complementation studies with these genes collectively suggest that a BarA-independent mechanism also activates UvrY. The model in Fig. 9 also depicts the finding that UvrY stimulates *barA* expression, indicative of a positive autoregulatory loop within this system. Finally, SdiA activates *uvrY* expression, and in this way affects *csrB* expression. Although the sigma factor RpoS or sigma^{S} is important in stationary phase regulation, and *rpoS* transcription appears to be activated by *barA*, neither CsrA protein nor CsrB RNA levels were significantly affected by *rpoS* disruption.

[0030] The UvrY/BarA two component signal transduction system was recently recognized in *E. coli*, and biochemical and genetic evidence was presented to demonstrate direct phosphotransfer from BarA to UvrY. BarA of *E. coli* appears to be involved in the bacterial adaptive responses against hydrogen peroxide-mediated stress by activating transcription of the *rpoS* gene, which encodes a sigma factor involved in the expression of stationary phase and stress response genes.

[0031] A sequence which overlaps the –35 region of the *uvrY* promoter is a SdiA box that is important to activation of *uvrY* transcription. *E. coli*, *Salmonella* and *Klebsiella* spp. are not known to synthesize the AHL and have no apparent AHL synthase genes. Nevertheless, SdiA may provide a means of recognizing other species (e.g. within the intestinal tract) and modulating *csrB* expression through effects on *uvrY*. However, in studies of the *Salmonella sdiA* gene, the most active AHL derivative had no effect on the expression of the disclosed *csrB-lacZ* fusion in *E. coli*.

[0032] CsrA is a repressor of biofilm formation, while CsrB activates biofilm formation. The gratuitous induction of *csrA* in a preformed biofilm caused it to disperse by liberating viable planktonic cells. The effect of CsrA on biofilm formation was mediated largely through its role as a regulator of intracellular glycogen synthesis and turnover in the stationary phase of growth. The most striking finding noted upon examination of biofilm formation by strains in the present study was that ectopic expression of *uvrY* caused several-fold increase in biofilm formation, which was almost as great as the increase caused by a *csrA* mutation (Fig. 8). UvrY was able to activate biofilm formation independently of CsrB. The *gacA* gene of *Pseudomonas aeruginosa*, which is homologous to *uvrY*, appears to activate biofilm formation. A number of possible factors that are required for biofilm formation were examined, including twitching and swarming motililty, alginate biosynthesis, and autoinducer production, but none accounted for the regulatory effect of GacA.

[0033] In an embodiment of the invention there is provided a method of reducing biofilm formation by increasing CsrA levels in a bacterial strain. In some instances, the bacterial strain is an *E. coli* strain, a *Salmonella* strain, a *Klebsiella* strain or a related gamma proteobacteria. CsrA activity may be increased by decreasing transcription of csrB, by increasing transcription of csrA DNA, by reducing translation of csrA mRNA, or by reducing the half-life of csrA RNA and/or CsrA.

[0034] Agents causing increased CsrA activity are referred to herein as "CsrA stimulators", and include inhibitors of csrB transcription, and stimulators of CsrA transcription and/or translation.

[0035] In an embodiment of the invention there is provided a method of increasing biofilm formation by decreasing CsrA levels in a bacterial cell. In some instances, the bacterial cell will be an *E. coli* cell, a *Salmonella* cell, a *Klebsiella* cell or a related gamma proteobacteria. CsrA levels may be decreased by increasing the level of csrB transcripts in the bacterial cell.

[0036] As used herein, the level of RNA or protein is "increased" if it is at least 10% higher than is found in normal cells of the same type under the same growth conditions.

[0037] In an embodiment of the invention there is provided a method of increasing CsrB levels in a bacterial cell by increasing the levels of active UvrY in the cell. Levels of active UvrY in the cell may be increased by increasing the expression of one or both of BarA and/or SdiA, by increasing uvrY transcription or uvrY RNA translation, by increasing the rate of UvrY phosphorylation, by decreasing the rate of UvrY dephosphorylation, or by increasing the half-life of uvrY mRNA and/or UvrY in the cell.

[0038] In an embodiment of the invention there is provided a method of modulating biofilm formation in a bacterial cell, comprising modulating the level of active UvrY in the cell. In some instances, the bacterial cell is an *E. coli* cell. In some instances, the cell is a *Salmonella* cell or a related gamma proteobacteria.

[0039] In an embodiment of the invention there is provided a modulator of CsrA activity in a bacterial cell, said modulator comprising a nucleotide sequence containing the element CAGGAUG. The element may be repeated preferably from about 2 to about 100 times. In some instances, repeats of between 5 and 50 elements will be desirable. In some instances, repeats of 18 elements will be desirable. In some instances, repeats of 19 elements will be required.

[0040] In an embodiment of the invention there is provided a method of increasing biofilm formation in a bacterial cell, comprising inducing in that cell the expression of a repeated nucleotide sequence, said sequence being CAGGAUG (SEQ ID NO: 1).

[0041] In an embodiment of the invention there is provided a method of reducing biofilm formation, comprising increasing BarA levels in a bacterial cell. In some instances, the bacterial cell is preferably an *E. coli* cell, *Salmonella* cell, or a related gamma proteobacteria.

[0042] In an embodiment of the invention there is provided a method of decreasing biofilm formation comprising modulating UvrY phosphorylation.

[0043] In an embodiment of the invention there is provided a method of modulating biofilm formation in a bacterial cell, comprising modulating the level of phosphorylation of UvrY in the cell. In some instances, the cell is preferably and *E. coli* or *Salmonella* cell or a related gamma proteobacteria.

[0044] In an embodiment of the invention there is provided a modulator of biofilm formation in a bacterial cell, said modulator comprising an amino acid sequence selected from a portion of the amino acid sequence of CsrA shown to selectively bind RNA of biofilm-related genes under stringent conditions *in vitro*.

[0045] In an embodiment of the invention there is provided a method of modulating the expression of an RNA containing at least one of the nucleotide sequences UGCACACRRNYYGUGUGUG (SEQ ID NO: 2), UGCACACYYNRRGUGUGUG (SEQ ID NO: 3), UGCACACGGAUUGUGUGUG (SEQ ID NO: 4), and RNA sequences at least 90% homologous to at least one of these sequences, wherein Y represents a pyrimidine, R represents a purine and N represents either a purine or a pyrimidine, comprising providing an RNA binding agent specifically recognizing the sequence.

[0046] In some instances the RNA binding agent is a peptide or protein selected from a portion of the amino acid sequence of CsrA shown to selectively bind RNA of biofilm-related genes under stringent conditions *in vitro*, or an amino acid sequence at least 80% homologous thereto which specifically binds the same nucleotide sequence with at least 50% of the affinity of the amino acid sequence. In some instances the RNA binding agent is a small molecule having a binding surface sharing substantially the same hydrophobicity, charge distribution, hydrogen-bonding ability, Van der Waal's size and shape as the portion of the amino acid sequence which binds the nucleotide sequence, and which specifically binds the nucleotide sequence with at least 50% of the affinity of the amino acid sequence.

[0047] In an embodiment of the invention there is provided a method of identifying modulators of biofilm formation. This method comprises identifying agents which modulate the level of CsrA in a bacterial cell, such as an *E. coli* cell, *Salmonella* cell, or a related gamma proteobacteria. In one embodiment of this method, there is provided a reporter gene system approach to identifying inhibitors, comprising a fused nucleotide containing genetic material encoding a reporter (such as lacZ) fused to the regulatory region of the biofilm formation modulating gene of interest (such as uvrY, csrB, csrA, sdiA, and/or barA). In another embodiment of the method there is provided a mobility shift approach to identifying inhibitors of CsrA, comprising identifying agents which slow the migration of purified CsrA through a matrix which retards mobility based on molecular weight or size. In another embodiment of the method there is provided a transcriptional activation assay useful in identifying inhibitors of compounds upstream of csrB, comprising assaying *in vitro* activation of csrB expression by assaying csrB RNA levels.

[0048] Inhibitors of particular interest include kinase inhibitors, as well as small molecules having surfaces available for interaction with a similar Van der Waals radius, charge, hydrogen bonding affinity and shape as known interaction domains of UvrY, CsrB, SdiA or CsrA.

[0049] In an embodiment of the invention there is provided an inhibitor of CsrA activity, comprising a nucleotide containing two or more repeats of the sequence element CAGGAUG.

[0050] In an embodiment of the invention there is provided a stimulator of biofilm formation, comprising a nucleotide containing two or more repeats of the sequence element CAGGAUG.

[0051] In an embodiment of the invention there is provided a modulator of biofilm formation, comprising an amino acid selected from a portion of the amino acid sequence of CsrA shown to selectively bind RNA of biofilm-related genes under stringent conditions *in vitro*

[0052] In an embodiment of the invention there is provided a modulator of biofilm formation, comprising a small molecule having a binding surface sharing the shape, hydrophobicity, hydrogen bonding affinity and charge distribution of the CsrA region which binds to flhDC transcripts.

[0053] In an embodiment of the invention there is provided a modulator of biofilm formation, comprising a small molecule having a binding surface sharing the shape, hydrophobicity, hydrogen bonding affinity and charge distribution of the CsrA region which binds to glgCAP transcripts.

[0054] In an embodiment of the invention there is provided a method of modulating glycogen biosynthesis and catabolism comprising modulating the level of CsrA in a bacterial cell. In some instances, the cell is preferably and *E. coli* or *Salmonella* cell, or a related gamma proteobacteria.

[0055] In an embodiment of the invention there is provided a method of modulating glycogen biosynthesis, comprising inducing the presence within a bacterial cell of a nucleotide having two or more repeats of the sequence CAGGAUG.

[0056] In an embodiment of the invention there is provided a method of modulating glycogen biosynthesis in a bacterial cell comprising administering to the cell a compound having an RNA binding region sharing substantially the same shape, size, charge distribution, and hydrogen bonding affinity as an amino acid sequence selected from a portion of the amino acid sequence of CsrA shown to selectively bind RNA of biofilm-related genes under stringent conditions *in vitro in situ* and which specifically binds RNA sequence UGCACACRRNYYGUGUGUG, UGCACACYYNRRGUGUGUG, UGCACACGGAUUGUGUGUG, or RNA sequences at least 90% homologous to at least one of these sequences, wherein Y represents a pyrimidine, R represents a purine and N represents either a purine or a pyrimidine, under physiological conditions with at least 50% of the affinity of CsrA for this RNA sequence.

[0057] In an embodiment of the invention there is provided a method of improving recovery of a mammalian patient suffering from infection by bacteria forming biofilm, comprising administering to the patient in a form suitable for uptake by the bacteria, an inhibitor of biofilm formation. In some instances, the bacteria is preferably an *E. coli* or *Salmonella* bacteria, or a related gamma proteobacteria. In some instances, the patient is a human or domestic animal.

[0058] In an embodiment of the invention there is provided a method of reducing bacterial load in a mammalian patient suffering from infection by bacterial forming biofilm, comprising administering to the patient, in a form suitable for uptake by the bacteria, a compound having an RNA binding region sharing substantially the same shape, size, charge distribution, and hydrogen bonding affinity as a sequence selected from a portion of the amino acid sequence of CsrA shown to selectively bind RNA of biofilm-related genes under stringent conditions *in vitro in situ* in CsrA, and which specifically binds UGCACACRRNYYGUGUGUG, UGCACACYYNRRGUGUGUG, UGCACACGGAUUGUGUGUG, or RNA sequences at least 90% homologous to at least one of these sequences, wherein Y represents a pyrimidine, R represents a purine and N represents either a purine or a pyrimidine, with at least 50% of the affinity of CsrA for this sequence.

[0059] Agents may be placed into a form suitable for uptake by bacteria by encapsulation using membranes selected to encourage uptake by bacteria, or other known means.

[0060] In an embodiment of the invention there is provided a modulator of biofilm formation by a bacterial cell comprising an agent having an AHL binding domain and a DNA binding domain and having at least 50% of the activity of SdiA in stimulating UvrA under physiological conditions.

[0061] In some instances the AHL binding domain is an amino acid sequence between 80% and 99% homologous to the SdiA DNA binding domain. In some

instances the DNA binding domain is an amino acid sequence between 80% and 99% homologous to the SdiA DNA binding domain.

EXAMPLES

Materials and Methods

[0062] Strains, plasmids and phage. The bacterial strains, plasmids, and bacteriophage used in this study are listed in Table 1.

[0063] Media and growth conditions. Luria-Bertani medium (34) was used for routine cultures. Kornberg medium (1.1% K_2HPO_4, 0.85% KH_2PO_4, 0.6% yeast extract containing 0.5% glucose for liquid medium) was used to grow cultures for the gene expression assays and Northern blot analysis. M63 medium supplemented with glucose (0.4%), thiamine (5 µg/ml), adenine and thymine (50 µg/ml), calcium pantothenate (1 µg/ml), and serine, glycine, methionine (100 µg/ml each), was used for the selection of *rel*$^+$ *barA* mutants (55). Tryptone broth (pH 7.4) contained 1% tryptone and 0.5% NaCl. Colonization factor antigen (CFA) medium (pH 7.4) contained 1% casamino acids, 0.15% yeast extract, 0.005% $MgSO_4$, and 0.0005% $MnCl_2$ (16). The following antibiotics were added, as required, at the following concentrations: chloramphenicol, 20 µg/ml; kanamycin, 50 µg/ml; ampicillin, 100 µg/ml; and tetracycline, 10 µg/ml, except that ampicillin and kanamycin were used at 50 and 40 µg/ml, respectively, during the construction of the *uvrY*-'*lacZ* fusion, and kanamycin was used at 100 µg/ml for the selection of *csrA::kanR* strains. All cultures that were used for gene expression assays were grown at 37°C with rapid rotary shaking (48).

[0064] Molecular and genetic techniques. P1*vir* transduction or cotransduction of resistance markers, subcloning, PCR amplification, and molecular genetic techniques were performed by standard procedures (34, 50).

[0065] The plasmid pBarA was constructed by PCR amplification of the *barA* gene from -270 to the end of the *barA* coding region from *E. coli* MC4100 chromosomal DNA using Pfu polymerase and the primers 5'-GAGAATGCATACGCCAAAATGAGGACAG (SEQ ID NO: 5) and 5'-GCGGATCCACTCGACAAGACATCCATTA (SEQ ID NO: 6). The resultant product was cloned directly into the pGEM-T vector (Promega) using the T-overhang, with the *barA* gene position in a clockwise direction. A 0.5-kb *Bam*HI-*Eco*RI fragment containing *csrA* from pCSR10 (48), a 1.3 kb *Eco*RI-*Hin*dIII fragment containing *uvrY* from pCA9505 (36), and a 3.0-kb *Nco*I-*Not*I fragment containing *barA* from pBarA were treated with the Klenow fragment of DNA polymerase I and were individually subcloned into the blunt-ended *Vsp*I site of pBR322 to generate pCRA16, pUY14, and pBA29, respectively. The open reading frames of the above genes are oriented in the same direction as *bla* in the vector.

[0066] Special precautions were required for two of the P1*vir* transductions of this study, as follows. The *barA::kanR* mutation was transduced from AKP014 (42) into MG1655 with selection for kanamycin resistance. Because AKP014 is a *relA* mutant, and *relA* is separated by only 1.4 Kb of DNA from *barA*, a *relA* wild type transductant was selected by virtue of its ability to grow on M63 supplemented medium, and was designated as BA MG1655. The *uvrY::cam* mutation was transduced from AKP023 (42) into CF7789. Because AKP023 is a *flhD* mutant, and *flhD* is located 21 Kb away from *uvrY*, a motile transductant was identified using the plate assay for motility, and designated as UY CF7789. The other *barA* or *uvrY* mutants were generated by P1*vir* transduction from BA MG1655 or UY CF7789, respectively.

[0067] Construction of a chromosomal *uvrY'-'lacZ* translational fusion. A 572-bp fragment containing the upstream regulatory region and 12 codons of *uvrY* was amplified from MG1655 DNA by PCR using the primers 5'-CAGCATCGCTTTCAGGCAGGAGACTTC (SEQ ID NO: 7) and 5'-CAGTTCGTGGTCATCAACAAGTAGAACG (SEQ ID NO: 8) and was treated with T4 DNA polymerase and polynucleotide kinase and subcloned into the *Sma*I site of

pMLB1034 (51). The resulting plasmid, pUZ9, contained 26 codons of *yecF*, which is upstream from and divergently oriented with respect to *uvrY* (7), the complete upstream flanking region of *uvrY*, 12 codons of the *uvrY* coding region, and an in frame *uvrY*-'*lacZ* translational fusion. DNA sequencing was performed to confirm the presence of the correct fusion and the absence of PCR-generated mutations. The *uvrY*-'*lacZ* fusion in pUZ9 was moved into the *E. coli* CF7789 chromosome using the lambda-InCh1 system as described (9). The resulting strain, KSY009, which was chosen for subsequent studies was Amp^r Kan^s and was no longer temperature sensitive. The presence of the *uvrY*-'*lacZ* translational fusion was confirmed by PCR analysis, as recommended (9). Oligonucleotide primers used in this study were synthesized by Integrated DNA Technologies Inc., Coralville, Iowa.

[0068] Motility assay. The plate assay was initiated by stabbing a colony from an overnight culture into semi-solid agar (tryptone broth or CFA medium solidified with 0.35% agar). The plates were kept in a humidified incubator at 30°C and examined at ~16 h of growth (59).

[0069] β-Galactosidase and total protein assays. β-Galactosidase activity was assayed in 10-min reactions, as described previously (46). Total protein was measured by the bicinchoninic acid method using bovine serum albumin as the protein standard (53).

[0070] Purification of His6-tagged UvrY. His6-tagged UvrY was purified as described previously, except that purified protein was dialyzed against 10 mM Tris-OAc (pH 8.0) containing 25% glycerol and concentrated in Centricon 10 units (Amicon) (42).

[0071] In vitro transcription-translation. Effects of UvrY protein on *csrB-lacZ* expression were examined using S-30 extracts prepared from a *uvrY* mutant strain (UYCF7789), as previously described (31, 48), except that reaction volumes were scaled down to 28 µl. Radiolabeled proteins were detected by fluorography using

sodium salicylate (10) and methionine incorporation into the LacZ polypeptide was quantified by liquid scintillation counting of H_2O_2-solubilized gel sections (48).

[0072] Quantitative biofilm assay. Overnight cultures were inoculated 1:100 into fresh medium. In the microtitre plate assay, inoculated cultures were grown in a 96-well polystyrene microtitre plate. Growth of planktonic cells was determined by absorbance at 600 nm or total protein assay. Biofilm was measured by discarding the medium, rinsing the wells with water (three times), and staining bound cells with crystal violet (BBL) (O'Toole, Mol. Microbiol. 30: 295 (1998)). The dye was solubilized with 33% acetic acid (EM Science, Gibbstown, N.J.), and absorbance at 630 nm was determined using a microtitre plate reader (DynaTech, Chantilly, Va.). For each experiment, background staining was corrected by subtracting the crystal violet bound to uninoculated controls. All comparative analyses were conducted by incubating strains within the same microtitre plate to minimize variability. To confirm that observed effects on biofilm formation in microtitre wells were not surface specific, cultures were grown and tested simultaneously in new borosilicate glass test tubes (18 mm).

[0073] Northern hybridization. RNA isolation, riboprobe synthesis and Northern blotting were conducted. Total cellular RNA (5 µg) was separated on formaldehyde agarose (1%) gels, transferred overnight onto positively charged nylon membranes (Boehringer Mannheim) in 20X SSC (1X SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and immobilized by baking at 120°C for 30 min (36). Prehybridization, hybridization to DIG-labelled riboprobes (2 µl of probe per 10 ml of prehybridization buffer), and membrane washing were conducted using the DIG Luminescent Detection kit for nucleotide acids (Boehringer Mannheim), according to the manufacturer's instructions. The resulting chemiluminescent signals were detected using Kodax X-Omat-AR film and were quantified by phosphorimaging using a GS-525 phosphorimager (Bio-Rad, Hercules, Calif.) with a chemiluminescent screen. Isolated CsrB RNA (23) was used to generate a standard curve for CsrB signal quantitation. Cellular CsrB transcript levels were quantified within the linear response range of the purified standard. Phosphorimaging data were analyzed

using Molecular Analyst version 2.1.2) software and Microsoft Excel. For RNA decay studies, strains were grown to the transition to stationary phase (A_{600} of 5.1 and 6.5 for the *csrA* wild type and mutant, respectively), rifampin (200 µg/ml [final concentration]) was added, and samples were collected at 0, 2, 4, 6, 8, and 12 min following rifampin addition. Samples (1.5 ml) were immediately centrifuged for 15 s at 15,000 X g, the spent medium was discarded, and the cells were frozen on dry ice-ethanol and stored at -80°C pending RNA isolation. RNA (5 µg) was separated on formaldehyde agarose (1.2 %) gels, blotted by capillary transfer onto positively charged nylon membranes (Boehringer Mannheim), and immobilized by baking at 120°C for 30 min. A digoxygenin (DIG)-labelled *csrB* riboprobe was synthesized from pSPT18-CsrB using the DIG Luminescent Detection kit for nucleic acids, as described by the manufacturer (Boehringer Mannheim). The blot was pre-hybridized and hybridized at a probe concentration of 50 ng/ml using Perfecthyb Plus™ hybridization buffer (Sigma Chemical). Signal detection used the commercial protocol (Boehringer Mannheim), except that incubation in blocking solution was extended for an additional 10 h. Chemiluminescent signals were detected using Kodak X-Omat-AR film. In addition, signals were quantified by phosphorimaging using a GS-525 Phosphorimager (Bio-Rad). Phosphorimaging data were analyzed using Molecular Analyst (version 2.1.2) software (Bio-Rad). Prior to blotting, the gels were stained with ethidium bromide and rRNA bands were photographed. The resulting signals were quantified by densitometry, and values used to correct for minor variations in sample loading.

Example 1: Effects of *uvrY* and *barA* on the in vivo expression of *csrB*.

[0074] Northern hybridization was used to determine if UvrY or BarA affect CsrB RNA levels (Fig. 1). RNA was isolated from MG1655 and its isogenic *uvrY* and *barA* mutants at ~2 h post-exponential phase, which is optimal for CsrB accumulation. CsrB RNA levels were decreased ~60% in the *barA* mutant, and ~98% in the *uvrY* mutant, relative to the parent strain. These results indicated that UvrY is an important regulator of *csrB* expression and that BarA influences *csrB* expression to a lesser extent.

[0075] To further determine if *uvrY* and *barA* regulate expression of *csrA* and *csrB*, expression from chromosomal *csrA'-'lacZ* translational or *csrB-lacZ* transcriptional fusions was examined in wild type, *uvrY* or *barA* mutant strains. The specific β-galactosidase activity from the *csrA'-'lacZ* fusion was not altered by either mutation (Fig. 2A), while the activity from the *csrB-lacZ* fusion was dependent upon both *uvrY* and *barA* (Fig. 2B). The *uvrY* mutation reduced *csrB-lacZ* expression ~95% or 20-fold, whereas, the *barA* mutant exhibited a decrease of ~70%. BarA is a member of the subclass of tripartite sensor kinases and UvrY is the cognate response regulator for BarA. These results indicate that BarA-phosphorylated UvrY activates transcription of the *csrB* gene, and that either unphosphorylated UvrY can activate *csrB* expression to a lesser extent or that UvrY is activated by an alternative phosphoryl donor.

Example 2: Effects of *csrA* and *uvrY* on *barA* expression.

[0076] To further examine the regulatory interactions of the CsrA/CsrB and BarA/UvrY systems, the effects of CsrA and UvrY on *barA* expression were examined by monitoring expression of a chromosomal *barA-lacZ* transcriptional fusion in wild type, *csrA* or *uvrY* mutant strains. The wild type strains exhibited ~2-fold greater β-galactosidase activity than their isogenic *csrA* (Fig. 3A) or *uvrY* mutants (Fig. 3B). Because HS703 was kanamycin resistant, this *csrA* isogenic strain pair was constructed by cotransduction of the *csrA::kanR* mutation along with a closely-linked *tetR* marker from TR1-5 CAG18642 into HS703 (*barA-lacZ*). Tet^r transductants were screened for the *csrA* glycogen phenotype to distinguish *csrA* wild type and mutant colonies, and isogenic *csrA* mutant and wild type strains, which each contained the *tetR* marker that was used for cotransduction, were compared in the assays (Fig. 3A). The *tetR* mutation itself increased *barA-lacZ* expression ~2-fold (compare the isogenic parent strains, CAGHS703 and HS703 in Fig. 3A and B). The gene disrupted by the *tetR* marker is *srlD* (*gutD*) and encodes the glucitol-6-phosphate dehydrogenase of the glucitol operon. The basis of its effect on *barA* expression is unknown. In conclusion, UvrY and CsrA each exhibit modest stimulation of *barA* expression.

Example 3: Effects of *csrA*, *barA*, and *uvrY* on *uvrY* expr ssion.

[0077] Expression from a *uvrY'-'lacZ* translational fusion in *csrA* wild-type and mutant strains was examined. Expression from this gene fusion was not affected by a *csrA* mutation. Overexpression of *csrA* from a plasmid resulted in only slight activation of this fusion, which is not likely to be biologically relevant. Similarly, the disruption of *barA* or *uvrY* had no effect on the expression of this *uvrY'-'lacZ* fusion.

Example 4: Complementation studies: effects of ectopic expression of *csrA*, *uvrY* or *barA* on *csrB-lacZ* expression in *csrA*, *uvrY*, and *barA* mutants.

[0078] CsrA is a strong activator of *csrB* transcription. This effect is indirect. *uvrY*, and *barA* activate *csrB* expression (Figs. 1 and 2), and *csrA* modestly stimulates *barA* expression (Fig. 3). Complementation studies were conducted to further delineate the regulatory circuitry of this system. Multicopy plasmids containing either *csrA* or *uvrY* restored *csrB-lacZ* expression in a *csrA* mutant background (Fig. 4). Only *uvrY* could restore *csrB-lacZ* expression in a *uvrY* mutant (Fig. 4). *barA, uvrY* or *csrA* were able to enhance *csrB-lacZ* expression in a *barA* strain background (Fig. 4). These results suggest a late or terminal role for UvrY in a signalling pathway to *csrB*. These results indicate that *csrA* influences *csrB* expression through BarA-dependent and independent mechanisms. In addition, *csrA* has no effect on *csrB* expression in the *uvrY* mutant background, indicating that its role in *csrB* expression is completely dependent upon UvrY. While the invention is not limited to any particular mechanism, this suggests that CsrA is involved in both BarA-dependent and -independent pathways for UvrY activation. Taken in context, the failure of *barA* to complement a *csrA* defect also suggests that CsrA may affect BarA function, e.g. CsrA may indirectly influence BarA activation.

Example 5: Effects of *uvrY* and *barA* on CsrB-activated genes.

[0079] Expression of a chromosomal *glgCA'-'lacZ* translational fusion and a plasmid-encoded *glgC'-'lacZ* translational fusion were monitored. The effect of *uvrY*

disruption was similar to that of *csrB*, and resulted in a modest decrease in *glgCA'-'lacZ* expression (Fig. 5A). Likewise, overexpression of *uvrY* yielded the opposite effect of *uvrY* and *csrB* disruption (Fig. 5B). Ectopic expression of *uvrY* from the multicopy plasmid pUY14 in a *csrB* null mutant strain resulted in modest to negligible effects on *glgCA'-'lacZ* (Fig. 5C), suggesting that UvrY affects expression of *glgCAP* primarily through its role as an activator of *csrB*. The *uvrY* and *barA* mutations also decreased *glgC'-'lacZ* expression from pCZ3-3 ~2-fold.

Example 6: UvrY activates *csrB* expression in vitro.

[0080] In vitro transcription-translation of pCBZ1-encoded *csrB-lacZ* transcriptional fusion was examined in S-30 extracts prepared from the *uvrY* mutant, UY CF7789, in the presence of various concentrations of purified UvrY protein. The expression of the pCBZ1-encoded *csrB-lacZ* fusion was activated ~20-fold by *uvrY* in vivo, as determined in *uvrY* wild type versus mutant strains. As shown in Fig. 6, in vitro synthesis of the LacZ protein was stimulated ~6-fold in the presence of 2.3 μM UvrY protein subunits, a concentration that saturated the reaction. Synthesis of the LacZ polypeptide was also detected in reactions with the control vector, pGE593. This was likely due to read-through transcription. However, in contrast to the reactions with pCBZ1, LacZ expression from the control vector was not stimulated by UvrY. Since pCBZ1 contained the upstream region of *csrB* (–242 to + 4), this indicates that UvrY activates *csrB* transcription, presumably by binding to *csrB* DNA. In this experiment, recombinant UvrY protein was used as isolated from the cell. Since BarA is required for maximal expression of *csrB* in vivo (Figs. 1 and 2), it is likely that phosphorylated UvrY activates *csrB* transcription. UvrY protein may be phosphorylated prior to or during the S-30 reaction, or that unphosphorylated UvrY may bind to DNA and activate transcription, albeit at reduced affinity relative to the phosphorylated form.

Example 7: Effects of *sdiA* on expression of *csrA*, *csrB*, and *uvrY*.

[0081] SdiA is a LuxR homologue that possesses a putative AHL binding domain and a second domain for binding DNA. Genomic array studies indicated that an increase in the copy number of *sdiA* significantly increases the levels of *uvrY* mRNA. Expression from chromosomal *csrA*'-'*lacZ*, *csrB-lacZ*, and *uvrY*'-'*lacZ* fusions in isogenic *sdiA* mutant and wild type strains was examined. Expression from these fusions in strains containing a plasmid clone of *sdiA*, pSdiA, or the cloning vector, pBR322 was also compared. No significant effect of SdiA was observed for *csrA*'-'*lacZ* expression (Fig. 7A, B). However, expressions from *csrB-lacZ* and *uvrY*'-'*lacZ* fusions were partially dependent upon *sdiA,* as they were decreased by the *sdiA* mutation (Fig. 7C, E) and increased 1.5- fold and ~6-fold, respectively (Fig. 7D, F), by *sdiA* overexpression. In order to determine whether *sdiA* regulates *csrB* expression via its effect on *uvrY*, a complementation test was conducted. Expression from the *csrB-lacZ* fusion in the *uvrY* mutant was no longer affected by *sdiA* overexpression, suggesting that *sdiA* stimulates *csrB* expression through its effects on *uvrY*. No effect of AHL, over a broad concentration range, on *lacZ* fusions for *csrA, csrB* or *uvrY*, in *sdiA* wild type or mutant strains was observed.

Example 8: Effects of *uvrY, barA*, or *sdiA* on biofilm formation.

[0082] CsrA represses biofilm formation, while CsrB activates this process. The effects of *uvrY*, *barA*, and *sdiA* on biofilm formation were monitored in static cultures using the microtiter plate assay, which measures the binding of crystal violet to adherent cells of the biofilm. The ectopic expression of *uvrY* activated biofilm formation several-fold (Fig. 8B). This effect was almost as great as that of a *csrA* knockout mutation. In addition, *uvrY* overexpression activated biofilm formation in a *csrB* mutant strain background, indicating that UvrY has effects on biofilm formation that are mediated independently of *csrB*. More modest effects were observed for knockouts of *csrB*, *uvrY, barA* and *sdiA*, which were still statistically significant (Fig. 8A). The parent strain (MG1655) formed ~3-fold more biofilm than each of these

mutants. Overexpression of *barA* caused a modest decrease in biofilm formation. The increased gene dosage of *sdiA* caused a modest increase in biofilm formation.

[0083] Thus, there has been provided compounds and methods for modulating bacterial functions.

TABLE 1. Bacterial strains, plasmids, and phages

Strain, plasmid, or phage	Description
Strains	
AKP014	MC4100 *barA*::*kanR*
AKP023	MC4100 *uvrY*::*cam*
BA MG1655 [a]	MG1655 *barA*::*kanR* (from AKP014)
CAG18642	*zfh*-3131::Tn*10*; 57.5 min, (near *csrA*)
CF7789	MG1655 Δ*lacIZ* (*Mlu*I)
DHB6521	SM551 λInCh1 (Kan^r)
HS703	MC4100 *barA*::λ*plac*Mu53 [Φ(*barA-lacZ*)*1010*]
KSA712	CF7789 Δ(*att-lom*)::*bla* Φ(*csrA*'-'*lacZ*)*1*(Hyb) Amp^r Kan^s
KSB837	CF7789 Δ(*att-lom*)::*bla* Φ(*csrB-lacZ*)*1*(Hyb) Amp^r Kan^s
KSY009	CF7789 Δ(*att-lom*)::*bla* Φ(*uvrY*'-'*lacZ*)*1*(Hyb) Amp^r Kan^s
KSGA18	CF7789 Φ(*glgA*::*lacZ*) (λ*plac*Mu15)
MG1655	Prototrophic
RG1-B MG1655 [a]	*csrB*::*cam*
SM551(=DHB6501)	F^- λ^- λ^s Δ*lac*(*MS265*) *mel* $NalA^r$*supF58* (=*sulII*$^+$)
TR1-5 MG1655[a]	*csrA*::*kanR*
UY CF7789 [a]	CF7789 *uvrY*::*cam* (from AKP023)
WX2	Δ*lac-pro met pro zzz*::Tn*10 thy supD* r_K^- m_K^- *sdiA*::*kanR*
Plasmids	
pCBZ1	pGE593 Φ(*csrB-lacZ*)

pCZ3-3	pMLB1034 Φ*glgC*'-'*lacZ*)
pUZ9	pMLB1034 Φ (*uvrY*'-'*lacZ*)
pCSR10	Minimal *csrA* in pUC19
pCRA16	*csrA* in blunt-ended *Vsp*I site of pBR322, Tet^r
pCA9505	Carries *uvrY* gene, Amp^r
pUY14	*uvrY* in blunt-ended *Vsp*I site of pBR322, Tet^r
pBarA	*barA* in pGEM-T, Amp^r
pBA29	*barA* in blunt-ended *Vsp*I site of pBR322, Tet^r
pSdiA	*sdiA* in *Eco*RI site of pBR322, Amp^r Tet^r
pSPT18-CsrB	Plasmid for the *csrB* riboprobe synthesis
pGE593	Vector for *lacZ* transcriptional fusions; Amp^r
pMLB1034	Vector for *lacZ* translational fusions; Amp^r
pGEM-T	T-cloning vector, Amp^r
pBR322	Cloning vector; Amp^r Tet^r

Bacteriophages

P1*vir*	Strictly lytic P1
λInCh1	For genomic insertions; Kan^r

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[a]Strain designations containing the prefix TR or TR1-5, RG or RG1-B, UY, BA, or SA indicate that the mutant allele *csrA*::*kanR*, *csrB*::*cam*, *uvrY*::*cam*, *barA*::*kanR*, or *sdiA*::*kanR*, respectively, was introduced by P1*vir* transduction.

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